

Remarks

I. Status and Nature of the Invention

Claims 1-95 were originally presented. Claims 1-4, 15, 21, 22 and 56-95 have been previously canceled. Claims 9, 10, 54, and 55 are canceled herein. Accordingly, claims 5-8, 11-14, 16-20, and 23-53 are presently pending. Claims 23-37 are withdrawn from consideration as directed to a non-elected species.

Applicants have amended claim 5 in order to more clearly recite that the assay method is a cell based assay wherein cells remain intact at the conclusion of the assay. Support for such an amendment can be found throughout the specification (see, for example, the published application US 2003/0077569 A1, paragraph 0013, third sentence). Claim 5 has also been amended in order to specify that a mixture composition comprising a substrate of the enzyme or an analyte compound and an agent that enhances uptake of said substrate or analyte compound is added to the cell sample to form a layer of the mixture composition over the cells. Support for such an amendment can be found, for example, in the last paragraph of Example 1 (paragraph 0149 of the published application US 2003/0077569 A1).

II. The Rejections Pursuant to 35 U.S.C. § 112, Second Paragraph

Claims 9, 10, 54, and 55 have been rejected pursuant to 35 U.S.C. §112, second paragraph as being indefinite. Although Applicants respectfully traverse the rejection, claims 9, 10, 54 and 55 have been canceled herein to facilitate the allowance of other claims in the case.

Thus, the rejection of claims 9, 10, 54 and 55 as indefinite pursuant to 35 U.S.C. §112(b), second paragraph, has been obviated.

III. The Rejections Pursuant to 35 U.S.C. § 102

A. The Rejections in View of Wansink

Claims 5, 9, and 16-19 have been rejected pursuant to 35 U.S.C. §102(b) as anticipated by Wansink *et al.* (1993, *J. Cell Biol.* 122(2):283-293). Applicants respectfully traverse the rejection and request reconsideration.

Applicants respectfully submit that the claimed invention is not anticipated by the disclosure of Wansink. As the Examiner will appreciate, the claimed invention comprises treating cells with a substrate or analyte for an enzyme that is to be assayed in the presence of an agent that enhances the uptake of that substrate or analyte into the metabolically active cells, and then assaying intact metabolically active cells for enzymic activity. In this regard, Applicants have amended claim 5 to more clearly point out that this invention is a cell-based assay wherein the assay results are measured in cells that remain intact at the conclusion of the assay. Wansink does not disclose a cell based assay wherein results are measured in intact cells. Rather, in Wansink, the assay results are measured on extracellular *precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells (please see page 284, left hand column, section entitled “BrUTP Incorporation in Permeabilized Cells (Run-on Transcription)”).

Claim 5 has been amended to specify that a mixture composition, comprising (i) a substrate for an enzyme to be assayed or an analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed in Wansink.

Applicants, therefore, respectfully submit that the rejection of claims 5, 9, and 16-19 pursuant to 35 U.S.C. §102(b) as anticipated by Wansink may be properly withdrawn.

III. The Rejections Pursuant to 35 U.S.C. § 103(a)

A. The Rejections in View of Lucas

Claims 5, 11, 12 and 20 have been rejected pursuant to 35 U.S.C. §103(a) as obvious in view of Lucas *et al.* (U.S. Patent 5,698,411). The rejection is predicated on the concern that although Lucas fails to disclose an assay in which multiple enzyme assays are conducted, or in which DMSO concentrations of between 20% and 60% are employed, such modifications would have been obvious to those of ordinary skill. Applicants respectfully traverse the rejection and request reconsideration.

As amended herein, Claim 5 now recites that a mixture composition, comprising (i) a substrate for an enzyme to be assayed or analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed or suggested in Lucas. Although Lucas discloses a homogeneous reaction mixture containing 5% DMSO as a solubilizing agent, there is no disclosure or suggestion of the instant invention which is directed to the use of much higher concentrations of DMSO in a mixture with the substrate or analyte compound to provide a mixture of sufficient density so that a layer of the mixture will be formed over the cells. The methods of the invention increase the effective concentration of the substrate or analyte compound in proximity to the cells.

Accordingly, Applicants respectfully submit that the rejection of claims 5, 11, 12 and 20 pursuant to 35 U.S.C. §103(a) as obvious over Lucas may now be properly withdrawn.

B. The Rejections in View of Landrum in combination with Lucas

Claims 5-14, 20 and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Landrum *et al.* (U.S. Patent 5,976,822) in view of Lucas *et al.* (U.S. Patent 5,698,411). The rejection is predicated on the concern that although Landrum fails to disclose the use of uptake-enhancing agents, the inclusion of such additional ingredients would have been obvious to those of ordinary skill in light of the teaching of Lucas that

certain solubilizing agents can be employed in a cell-based enzyme assay. Applicants respectfully traverse the rejection.

As amended herein, Claim 5 now recites that a mixture composition, comprising (i) a substrate for an enzyme to be assayed or an analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed or suggested in either the Landrum or the Lucas reference. The assays in Landrum and Lucas were conducted in homogeneous reaction mixtures. Although Lucas discloses a homogeneous reaction mixture containing 5% DMSO as a solubilizing agent, there is no disclosure or suggestion in either Landrum or Lucas of the use of much higher concentrations of DMSO in a mixture with the analyte or substrate to provide a mixture of sufficient density that will form a layer over the cells.

Accordingly, Applicants respectfully submit that the rejection of claims 5-14, 20 and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Landrum in view of Lucas may now be properly withdrawn.

C. The Rejections in View of Zhang

Claims 5-14, 20, and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Zhang *et al.* (U.S. Patent 6,248,904). The rejection is predicated on the concern that although Zhang fails to disclose an assay in which multiple enzyme assays are conducted, or in which DMSO concentrations of between 20% and 60% are employed, such modifications would have been obvious to those of ordinary skill. Applicants respectfully traverse the rejection.

Additionally, the claims as amended herein recite that a mixture composition, comprising (i) a substrate for an enzyme that is to be assayed or an analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is

added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed or suggested in the Zhang reference. The assays in Zhang were conducted in a homogeneous reaction mixtures. Although Zhang discloses that assay mixtures may contain solubilizing agents such as DMSO, there is no disclosure or suggestion in Zhang of sufficiently high concentrations of DMSO in a mixture with the substrate or analyte compound to provide a mixture of sufficient density to form a layer over the cells.

Accordingly, Applicants respectfully submit that the rejection of claims 5-14, 20, and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Zhang may now be properly withdrawn.

D. The Rejections in View of Landrum. in combination with Lucas and Wansink

Claims 5-14, 16-20 and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Landrum *et al.* (U.S. Pat. No. 5,976,822), in view of Lucas *et al.* (5,698,411), and further in view of Wansink *et al.* (1993, *J. Cell Biol.* 122(2):283-293). The rejection is predicated on the concern that the Landrum/Lucas combination as discussed above does not disclose the use of glycerol as a solubilizing or permeabilizing agent. Thus, Wansink is cited in conjunction with Landrum/Lucas to provide the suggestion to use glycerol in the reaction mixtures.

Applicants respectfully traverse the rejection. As discussed above, Wansink discloses an assay procedure wherein the assay results are measured on extracellular *precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells rather than on intact cells as in the instant invention. Thus the methods disclosed in Wansink did not require the maintenance of cellular integrity as is required in the instant invention. Thus, one of skill in the art would not have been motivated to combine the teachings of Wansink with the teachings of Landrum/Lucas to arrive at the invention wherein glycerol is the uptake enhancing agent

Additionally, the claims as amended herein now recite that a mixture composition, comprising (i) a substrate for an enzyme that is to be assayed or an analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into cells, is added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed or suggested by any of Landrum, Lucas or Zhang.

Accordingly, Applicants respectfully submit that the rejection of claims 5-14, 16-20 and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Landrum, in view of Lucas and Wansink may now be properly withdrawn.

E. The Rejections in View of Zhang *et al.* in combination with Wansink

Claims 5-14, 16-20 and 38-54 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over Zhang *et al.* (U.S. Patent 6,248,904) in view of Wansink *et al.* (1993, *J. Cell Biol.* 122(2):283-293). The rejection is predicated on the concern that Zhang *et al.* does not disclose or suggest an assay in which glycerol was used as a solubilizing or permeabilizing agent. Thus, Wansink is cited in conjunction with Zhang to provide the suggestion to use glycerol in the assay mixtures.

Applicants respectfully traverse the rejection. As discussed above, Wansink discloses an assay procedure wherein the assay results are measured on extracellular *precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells rather than on intact cells as in the instant invention. Thus the methods disclosed in Wansink did not require the maintenance of cellular integrity as is required in the instant invention. Thus, one of skill in the art would not have been motivated to combine the teachings of Wansink, with the teaching of Zhang to arrive at the invention wherein glycerol is the uptake enhancing agent.

Additionally, the claims as amended herein now recite that a mixture composition, comprising (i) a substrate for an enzyme that is to be assayed or an analyte compound and

(ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to the cell sample so that the mixture composition forms a layer over the cells. The concept of a layered or non-homogeneous assay mixture was not disclosed or suggested by either of Zhang or Wansink.

Accordingly, Applicants respectfully submit that the rejection of claims 5-14, 16-20, and 38-54, pursuant to 35 U.S.C. §103(a), as obvious over Zhang in view of Wansink may now be properly withdrawn.

III. Concluding Remarks

Having now fully responded to all outstanding rejections, Applicants respectfully submit that the present application is in condition for Allowance, and earnestly solicit early notice of such favorable action. The Examiner is respectfully invited to contact the undersigned with respect to any issues regarding this application.

Respectfully Submitted,

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